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Development of Synthetic Catalysts for Peptide Bond Cleavage
Synthesis and Complete Kinetic Analysis of Compounds 6A, 7A, 8A

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19 ABSTRACT (Continue on reverse if necessary and identify by block number)

Synthetic mimics for carboxypeptidase A will be synthesized and the structural and chemical factors responsible for catalytic peptidase activity will be probed. Ditopic macrocyclic receptors have been designed which incorporate the salient features of the enzyme analog, namely high affinity complex formation, general base and general acid catalysis, and covalent catalysis. Once synthesized the resulting macrocycle-metal ion complexes should non-specifically promote the hydrolysis of C-terminal peptide bonds. The initial macrocycles will have several types of coordination sites: nitrogen-containing heterocycles, ammonium and ether oxygens. One side of the ditopic receptor will preferentially bind zinc(II) ion, the other the peptide substrate.

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FINAL REPORT ON CONTRACT N00014-86-K-0862

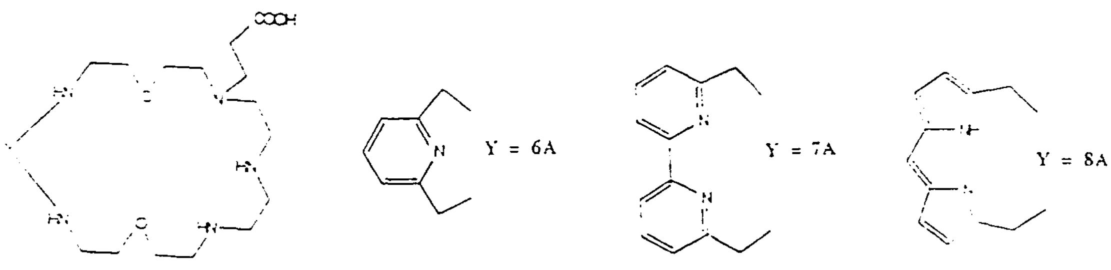
PRINCIPAL INVESTIGATOR: Kristin Bowman Mertes

CONTRACT TITLE: Development of Synthetic Catalysts for Peptide Bond Cleavage: Synthesis and Complete Kinetic Analysis of Compounds **6A**, **7A**, **8A**

START DATE: 6 August 1986

RESEARCH OBJECTIVE:

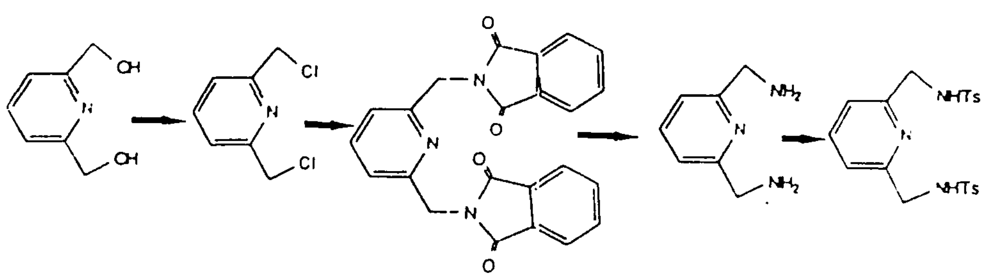
The synthesis of ditopic receptors **6A**, **7A**, **8A**, designed by considering the important interactive features of carboxypeptidase A (CPA) was the goal of this project. Three basic macrocyclic ligands with site specificity for zinc(II) incorporation and functionalized podando groups for covalent catalysis were attempted. Two of these were synthesized and examined for hydrolase activity. The design of the macrocycles was to allow for the critical assessment of the importance of the interactive sites within the natural enzyme, from the general acid catalysis provided by the arginine and tyrosine residues and the zinc(II) ion to the general base role of the Glu-270 residue.



ACCOMPLISHMENTS:

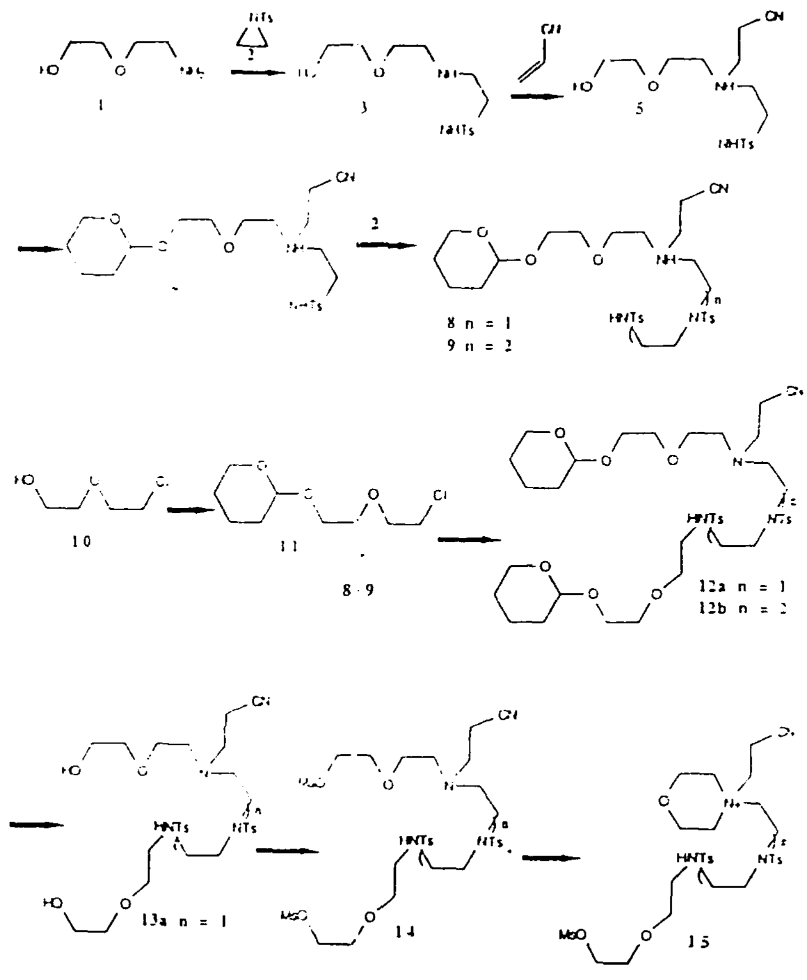
Synthesis

The synthetic efforts could be divided into the synthesis of the two halves of the molecule, the "eastern" and "western" half. The synthesis of the "western" half was relatively straightforward for compound **6A** and was accomplished in the first year of the contract (Scheme 1):

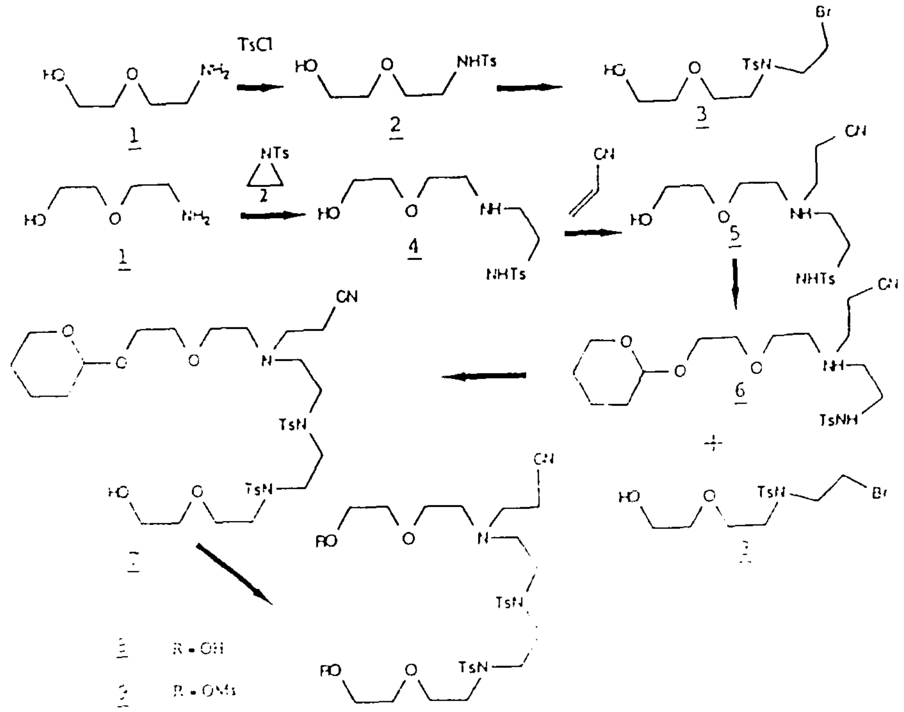


Scheme 1

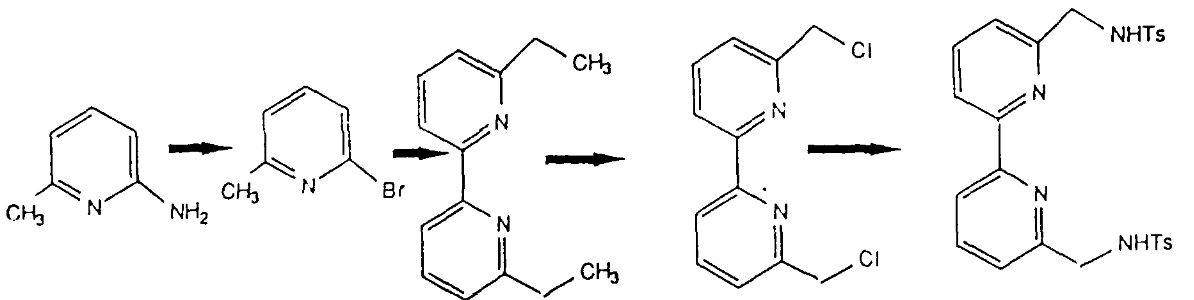
In the synthesis of the "eastern" half of the molecule several synthetic problems were encountered. One of the major difficulties was a polymerization reaction where more than one unit of the tosylaziridine added to the azoxy chain. This was further complicated by difficulties in separating the three major isomers. In proceeding from 7 to 8 the tosyl aziridine reacted further with the triamine product to give tetraamines and higher analogs. A second problem was found to occur in the mesylation of 13a, in which 14 was lost due to an undesired ring closure leading to 15.



These problems were solved in year two by using a convergent sequence as shown in Scheme 2. Here 3 and 6 are combined to give 7. This avoided the difficulties in extension of 5, which could not be controlled.



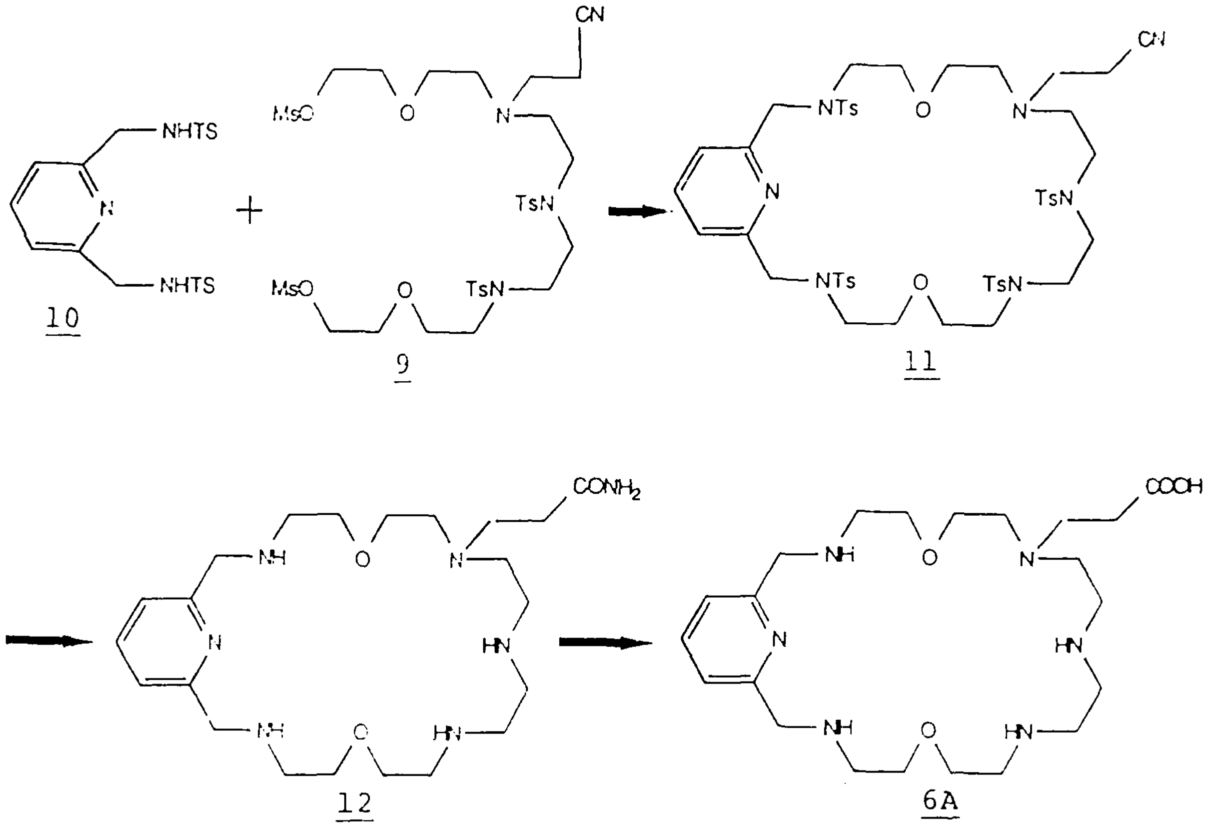
The "western" half of 7A was prepared as shown in Scheme 3, with low overall yield.



13 14 15 16

Scheme 3

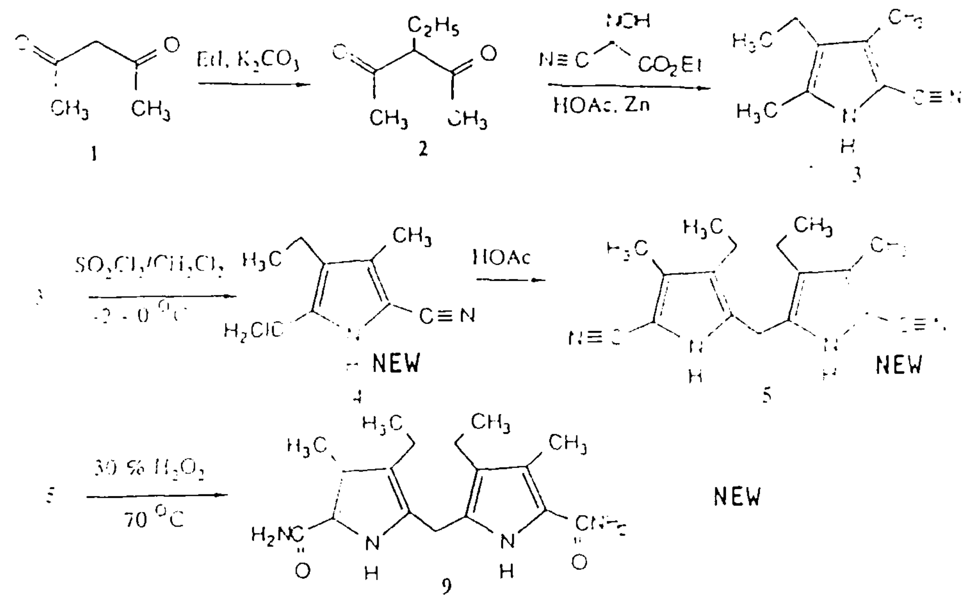
The target compounds 6A and 7A were pr pared as shown in Scheme 4 for 6a. The reaction of 10 with 9 gave a reasonable yield of the protected macrocycle 11. Deprotection of 11 was critical until precise reagent quantities and temperature control were found.



Scheme 4.

Testing for catalytic activity was completed on **6A** and **7A** and their precursur amides (e.g., 12, Scheme 4) using the hydrolysis of O-(trans-p-chlorocinnamoyl)-L-ß-phenyllactic acid at 80 ^{o}C and pH 7.0. The results are shown in Table 1. Only one of the compounds showed any evidence of catalytic activity, **6A**. These studies and related studies in phosphoryl transfer catalysis indicate that steric effects play a crucial role in the catalytic activity of these macrocycles. Hence, less sterically hindered substrates are more desirable for testing these compounds.

The synthesis of compound **8A** was not achieved due to the extreme sensitivity of the dipyrromethane portion of the macrocycle. Several new pyrrole and dipyrromethanes were synthesized (Scheme 5), however, and there chemistry is currently being investigated.



Scheme 5

While the compounds synthesized were not catalytically active, a variety of new synthetic techniques were achieved. The critical point in these studies to be made is that ring size must be optimal and steric hindrance minimal for any reaction to occur. These findings are corroborated by modeling studies in phosphoryl transfer reactions, where the macrocycles are only active for 21- and 24- membered ring systems, and catalysis is greatly reduced in the presence of podando side chains.

PUBLICATIONS AND REPORTS:

Gu, K.; Mertes, K. B.; Mertes, M. P., Strategy for the Synthesis of Unsymmetrical N-Substituted Polyazamacrocycles, Tetrahedron Lett. 1989, 30, 1323-1326.

Table 1. First Order Rate Constants (k_{obsd}) for the Hydrolysis of O-(trans-p-chlorocinnamoyl)-L-β-phenyllactic Acid at pH 7 and 80 °C.

macrocycle	metal	k_{obsd} (min^{-1}, x 10^5)
none	none	3.68
none	Zn^{2+}	4.99
24pyCONH2, 10	none	4.02
24pyCONH2, 10	Zn^{2+}	4.24
24pyCOOH, 6A	none	3.72
24pyCOOH, 6A	Zn^{2+}	5.82
27dipyCONH2, 11	none	2.96
27dipyCONH2, 11	Zn^{2+}	2.45
27dipyCOOH, 7A	none	2.50
27dipyCOOH, 7A	Zn^{2+}	2.65

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